Cardiovascular Disease, Diabetes and Atherosclerosis-Know Your Risk: Is it Time for a Paradigm Shift?

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Speaker Disclosure

I have received honoraria as a speaker for Boehringer Ingelheim/Eli Lilly, Janssen Pharmaceuticals, Inc., and Merck.

--Dr. Lardinois
OUR HEARTS AND PRAYERS
GO OUT TO VEGAS
Cardiovascular Disease and Diabetes: The Facts

- Cardiovascular complications are the leading causes of diabetes-related morbidity and mortality.
- Modification of traditional CVD risk factors, such as smoking cessation, managing diabetes, decreasing blood pressure, addressing psychological issues and obesity, healthier food choices, increasing physical activity, and lowering of cholesterol has resulted in reducing CVD (and stroke) especially in patients with diabetes.
- However, the current standard of care using traditional modifiable risk factors alone is frequently inadequate to identify some patients at risk for CVD and stroke.
- A new paradigm focusing on disease (atherosclerosis) is paramount; when disease is found the ‘root’ causes must be identified and treated aggressively.
Standard of Care
Addressing Modifiable Risk Factors
INTERHEART STUDY

- Smoking
- Diabetes (Insulin Resistance)
- Hypertension
- Psychosocial factors
- Abdominal obesity “apple”
- FORK-consumption of fruits and vegetables
- FOOT-physical exercise
- Alcohol intake
- Dyslipidemia
INTERHEART: Association of Risk Factors with Acute Myocardial Infarction in Men and Women

Adjusted for age, sex, geographic region
Note: odds ratio plotted on a doubling scale

Lancet 2004;364:937-952
INTERHEART STUDY

(+) Linear Relationship: Daily Cigarette Consumption and Myocardial Infarction

OR (99% CI)

Daily Cigarette Consumption

Lancet 2004;364:937-952
Insulin Resistance (IR)

- IR is a key player in promoting atherosclerosis
- IR is one of the cardinal features of CVD
- Components of metabolic syndrome, TG/HDL-C ratio, A1c, HOMA-IR, fasting glucose, fasting triglycerides and adiponectin may confirm IR, but have limitations

- **Gold Standard** OGGT (75 grams): one hour ≥ 125 mg/dL or two hour ≥ 120 mg/dL confirms IR
- Every person should be evaluated for IR!!
The 10 leading Causes of Death in the World, 2015

http://www.who.int/healthinfo/global_burden_disease/en
Psychosocial: Potential Pathways by which Psychosocial Factors Influence ASVD

**Psychosocial Stress Factors**
- External Stressors (life events, financial troubles)
- Chronic Stressors

**Health-Related Behaviors**
- Nutrition
- Physical Activity
- Smoking
- Alcohol

**Direct Pathophysiological Mechanisms***
- Atherosclerosis
- Plaque stability
- Coagulation
- Fibrinolysis

**Protective Factors**
- Income
- Education
- High locus of control

**Depression**

**Clinical ASVD**

Lancet 2004;364:937-952
Takotsubo Cardiomyopathy
Apical Ballooning

Broken Heart Syndrome

Circulation 2011;124:e460-e462
Deadly Duet
Foot and Fork Dysorder

Hyperactive Fork
To a mucha forka

Hypoactive Foot
Not Enuffa Foota
My doctor has limited me to only 2 beers a day!!
INTERHEART STUDY

(+) Linear Relationship: Apolipoprotein B/A-1 Ratio and Myocardial Infarction

OR (99% CI)

Deciles: 1 2 3 4 5 6 7 8 9 10

Lancet 2004;364:937-952
Despite Statin-Induced LDL-C Lowering Benefits, Treated Patients Still Have Substantial Residual Risk for CAD Events

Placebo Event Rates

Relative CVD Risk Reduction for Statin users (%)

Relative Risk for Statin users (%)

4S=Scandinavian Simvastatin Survival Study; CARE=Cholesterol and Recurrent Events; WOSCOPS=West of Scotland Coronary Prevention Study; LIPID=Long-term Intervention with Pravastatin in Ischemic Disease; AFCAPS=Air Force/Texas Coronary Atherosclerosis Prevention Study; HPS=Heart Protection Study; PROSPER=Prospective Study of Pravastatin in Elderly at RISK; CARDS=Collaborative Atorvastatin Diabetes Study; ASCOT-LLA=Anglo-Scandinavian Cardiac Outcomes Trial; JUPITER=Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; CAD=coronary artery disease.
Total cholesterol and LDL-Cholesterol levels are **HIGHER** in men and women with diabetes

FALSE!!
Total Cholesterol & LDL-Cholesterol Levels are **Indistinguishable** *

<table>
<thead>
<tr>
<th>Condition</th>
<th>Women with diabetes</th>
<th>Women without diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC ≥275</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>TG ≥200</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>VLDL-C ≥35</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>LDL-C ≥190</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>HDL-C ≤41</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

*NOTE major differences: Higher triglycerides and Lower HDL-C*
Men and women with diabetes have a higher cardiovascular disease risk compared to men and women without diabetes

TRUE!!
Dyslipidemia, Diabetes, and Cardiovascular Disease

Clearly, a lipid parameter other than LDL-C is the explanation for the increased cardiovascular disease in patients with diabetes.

**Remember** triglycerides are higher and HDL-C is lower in patients with diabetes.
Framingham Offspring Study: Relationship of LDL Particles (LDL-P) and LDL Cholesterol (LDL-C) to Levels of HDL Cholesterol (HDL-C) and Triglycerides (TG)

Lower HDL-C and/or higher TG is associated with higher LDL-P; LDL-C is deceptively low in these discordant settings.

Curr Athero Reports 2004;6:381-387
Discordance Between LDL-C and LDL-P↑ with the # of Metabolic Components

Mean adjusted total LDL-P and LDL-C

LDL-C (mg/dL)

0 1 2 3 4 5

# of metabolic syndrome criteria

LDL-P (nmol/L)

1,000 1,200 1,400 1,600 1,800 2,000
Women’s Health Study: TC/HDL-C, ApoB/A-1 and Triglycerides Best CVD Predictors

LDL Cholesterol is #11!
Insurance Underwriting for Dyslipidemia Look at the Total Cholesterol and TC/HDL-C Ratio to Determine CVD risk*

<table>
<thead>
<tr>
<th>Underwriting Risk</th>
<th>Total Cholesterol</th>
<th>TC/HDL-C Ratio</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred Plus</td>
<td>220</td>
<td>5</td>
<td>500</td>
</tr>
<tr>
<td>Preferred</td>
<td>240</td>
<td>6</td>
<td>500</td>
</tr>
<tr>
<td>Standard</td>
<td>300</td>
<td>8</td>
<td>500</td>
</tr>
</tbody>
</table>

*Note that there is NO mention whatsoever of LDL-C! When is the medical finally going to figure out that LDL-C is not the major determinant of CVD risk?

The Bulletin June 2009; p18-19
FDA and Total Cholesterol/HDL-C Ratio

The FDA refuses to accept the ratio as a valid cardiovascular endpoint despite the overwhelming scientific literature showing it is superior to LDL-C and non-HDL-C in predicting subsequent cardiovascular events.

So what are we suppose to do?
The Apo B-containing (non-HDL) Lipoprotein Family: All Atherogenic Lipoproteins

- LDL
- Intermediate Density Lipoprotein (IDL)
- VLDL /VLDL remnants
- Chylomicron remnants
- Lp(a)

Rationale for therapeutic lowering of ApoB lipoproteins: decreases the probability of an inflammatory response
ApoB and Cardiovascular Disease

Like the total cholesterol/HDL-C ratio, ApoB is superior to LDL-C and non-HDL-C in predicting subsequent cardiovascular events.

The goal for ApoB in a patient with CVD should be less than $60$ mg/dL (correlates to a LDL-C of $\sim70$ mg/dL).
Standard of Care
Non-Modifiable Risk Factors

• Age-Clonal Hematopoiesis of Indeterminate Potential (CHIP)
• Gender-Cross Sex Hormone Therapy (CSHT)
• Ethnic background
• Family history of heart disease
Clonal Hematopoiesis of Indeterminate Potential (CHIP)
CHIP Carries Associated with Increased Risk of CHD and Early Onset Myocardial Infarction

<table>
<thead>
<tr>
<th>ATVB and PROMIS</th>
<th>No. of Participants with Myocardial Infarction/No. at Risk</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>31/46</td>
<td>1.4 (0.7–2.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>TET2</td>
<td>12/13</td>
<td>8.3 (1.2–357.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>ASXL1</td>
<td>8/8</td>
<td>Undefined</td>
<td>0.02</td>
</tr>
<tr>
<td>JAK2</td>
<td>16/16</td>
<td>Undefined</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>20/22</td>
<td>6.9 (1.7–61.6)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

N Engl J Med 2017;377:111-121
Age Related Prevalence of Clonal Hematopoiesis of Indeterminate Potential

Is age truly a non-modifiable risk factor??
The Time in NOW for a Paradigm Shift in Treating CVD and Diabetes

- Identify and aggressively treat traditional modifiable risk factors
  **PLUS**
- Non-invasive testing
- Genetic testing
- Inflammatory biomarkers
- Others: NT-proBNP, IL-1β, Obstructive Sleep Apnea, Telomere length (micronutrients)
Disease (Atherosclerosis) Identification

Disease (+) events

Disease (-) events

No Disease
Non-Invasive Imaging

- Coronary Calcium Score (CCS)
- Carotid Artery Duplex Scan
- Carotid Intima Media Thickness (cIMT)
- Ankle/Brachial Index (ABI)
- Abdominal Ultrasound
Coronary Calcium Score (CCS)

- CCS documents presence of atherosclerosis
- Identifies patients at increased risk for cardiovascular disease and stroke
- Adds prognostic power to conventional risk stratification tools (Framingham)
- Alters therapeutic goal (lipids, blood pressure, etc.)
- Improve Compliance (Adherence)

Circulation 2013;127:e6-e245
Calcium Score $<11 = 0.11\%$ CVD Risk Annually $= 1\%$ 10 years!*

<table>
<thead>
<tr>
<th>Calcium Score</th>
<th>Plaque Burden</th>
<th>Probability of Significant CAD</th>
<th>Annual Cardiac Event Rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>No identifiable plaque or insignificant plaque burden</td>
<td>Very unlikely, $&lt;1%$</td>
<td>0.11% per year</td>
</tr>
<tr>
<td>11-100</td>
<td>Mild to moderate atherosclerotic plaque burden</td>
<td>Non-obstructive CAD most likely</td>
<td>2.1% per year</td>
</tr>
<tr>
<td>101-400</td>
<td></td>
<td>Non-obstructive CAD likely, although obstructive disease possible</td>
<td>4.1% per year</td>
</tr>
<tr>
<td>&gt;400</td>
<td>Extensive atherosclerotic plaque burden</td>
<td>High likelihood of at least one significant coronary stenosis</td>
<td>4.8% per year</td>
</tr>
</tbody>
</table>

*Do these individuals justify statin treatment regardless of their age?
Coronary Calcium Score Best Cardiovascular Risk Marker
Carotid Artery Duplex Scan
CAVEAT:
Coronary Calcium Score ZERO

LAD SOFT PLATE
Carotid Intima Media Thickness (cIMT) is an Excellent Clinical Tool to Find and Monitor Plaque

- cIMT can detect subclinical atherosclerosis and an accelerated atherosclerotic process.
- cIMT is an independent predictor of heart attack and stroke.
- cIMT is noninvasive, inexpensive, and repeatable without adverse effects.
- cIMT can be used to monitor the atherosclerotic disease process.
- cIMT adds prognostic power to conventional risk stratification tools (Framingham).

Circulation 2013;127:e6-e245
Finding the Plaque

Plaque is defined as a cIMT of >1.2 mm in either the common, bifurcation, or internal carotid artery.
Ankle-Brachial Index (ABI)

**A** Ultrasound device amplifies the sound of arterial blood flow

**B** Systolic pressure recorded in the brachial artery of the arm

**C** Sound of arterial blood flow located in ankle

**D** Systolic pressure sequentially recorded in the arteries of the ankle after each arterial flow is located
Abdominal Aortic Ultrasound

[Image of an ultrasound scan with measurements and labels]
Personalizing Medical Care

- Effective medical management demands setting goals based, as much as possible, on the biological uniqueness of each patient
- Biological differences are grounded in genetics
- Genetics will drive the future of medicine
- You cannot get any more personal than your genetic makeup
Genetic Testing for Atherosclerosis
YES I am Talking about you!

• ApoE genotype – lifestyle advice
• KIF6 Genotype – statin therapy
• 9p21 genotype – heart attack gene
• Haptoglobin Genotype – CV risk especially in people with type 2 DM
• Factor V Leiden-coagulation disorder
ApoE Genotype

• Three alleles (2, 3, 4) dominantly inherited
• ApoE is key in lipid metabolism; binding to receptors on liver and clearing chylomicrons and very-low density lipoproteins (VLDL)
• Linear relationship with total cholesterol and LDL-C 2/2, 2/3, 2/4, 3/3, 3/4, 4/4 (lowest to highest)
• Clinical relevance
  - risk for CVD and Alzheimer’s disease
  - lipoprotein metabolism
  - response to therapy
  - medical nutrition therapy

ApoE Genotype and Risk of CVD-
ApoE4/4 Highest Risk

JAMA 2007;298:1300-1311
Differences in Total Cholesterol and HDL-C by ApoE Genotype (Total Cholesterol/HDL-C ratio ↑’s)

JAMA 2007;298:1300-1311
ApoE4-Enhanced Binding Capacity to the LDL-Receptor vs. ApoE2

↓ LDL-C  ↑ LDL-C
## Medical Nutrition Therapy Critically Based on ApoE Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Apo E2 Response</th>
<th>Apo E3 Response</th>
<th>Apo E4 Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2/2</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Population Frequency</td>
<td>1%</td>
<td>10%</td>
<td>62%</td>
</tr>
<tr>
<td></td>
<td>3/4</td>
<td>2/4</td>
<td>3/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4/4</td>
</tr>
</tbody>
</table>
| Fish Oil\(^1\) | ↓ TG
↓ small dense LDL
↑ HDL | ↓ TG
↓ small dense LDL
↑ HDL | ↓ TG
↓ small dense LDL
↓ HDL
↑↑ LDL |
| Low Fat Diet\(^2,3\) | ↓ LDL
↑ small dense LDL | ↓ LDL
↔ small dense LDL | ↓ LDL
↓ small dense LDL
↓ HDL
↑↑ LDL |
| Moderate Fat Diet\(^3\) | ↔ LDL
↔ small dense LDL | ↓ LDL
↓ small dense LDL | ↓ LDL
↑↑ small dense LDL |
| Moderate Alcohol\(^4\) | ↑ HDL ↓ LDL | ↑ HDL | ↓ HDL ↑ LDL |

2. Am J Clin Nutr 2003;77:1098-1111
Foods Rich in Choline and L-Carnitine Accelerate Atherosclerosis

Food containing lecithin, choline, betaine and L-carnitine

Accelerate atherosclerosis

Gut microbes

trimethylamine

trimethylamine-N-oxide

TMAO

flavin monoxygenases

FMOs of liver

TMAO Reference Range <6.2 uM

Chin Med J 2015;128:2805-2811
Red Meat Consumption (Not SFA Intake) Increases All-Cause Mortality

Clinical Nutrition [http://dx.doi.org/10.1016/j.clnu.2017.06.013]
Kinesin-like Protein 6 (KIF6)

- Kinesins: a family of motor proteins which transport organelles, protein complexes and mRNAs within a cell
- The genetic variant changes tryptophan to an arginine KIF6Trp719Arg
- This change results in a non-polar residue replacing a basic residue in the tail domain which may affect cargo binding or regulation in the motor domain

Science 2003;302:2130-2134
In CARE, carriers of the 719Arg risk allele received significant reduction of absolute risk for CVD events from pravastatin therapy. No significant reduction was observed in noncarriers.

In WOSCOPS, CVD risk reduction was significantly greater in carriers than in noncarriers.

J Am Coll Cardiol 2008;51:435-443
PROVE IT-TIMI 22 Study

Carrier’s (+) BENEFIT

- High-dose atorvastatin
- Standard-dose pravastatin

HR = 0.59 (0.45-0.78)
P ≤ 0.001
Abs Risk Reduct = 10.0%
NNT = 10

Non-Carrier’s (-) BENEFIT

- High-dose atorvastatin
- Standard-dose pravastatin

HR = 0.98 (0.72-1.31)
P = 1.0
Abs Risk Reduct = 0.8%
NNT = 125

J Am Coll Cardio 2008;51:449-455
Science 2003;302:2130-2134
Clinical Significance of KIF6 Testing

• **KIF6 Carrier**
  - May have higher life time cardiovascular risk
  - **ANY** statin is beneficial

• **KIF6 Non-carrier**
  - Still can be at risk; monitor for disease
  - When considering statin therapy, choose simvastatin or rosuvastatin

• **KIF6 Status unknown**
  - Choose simvastatin or rosuvastatin
Atorvastatin Failed to Show Benefit in Females

Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA)

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>1.10 (0.57-2.12)</td>
<td>0.59 (0.44-0.77)</td>
</tr>
<tr>
<td>Male</td>
<td>0.59 (0.44-0.77)</td>
<td>0.64 (0.50-0.83)</td>
</tr>
<tr>
<td>All Patients</td>
<td>0.64 (0.50-0.83)</td>
<td>-</td>
</tr>
</tbody>
</table>

Lancet 2003;361:1149-1158
9p21

“Heart Attack Gene“

JAMA 2010;303:648-656
9p21 Risk Variant Alters the Activity of ANRIL Resulting in Increased Risk of Atherosclerosis and Aneurysm
### 9p21 Polymorphism Carriers have Increased Risk of CVD and Aneurysm

<table>
<thead>
<tr>
<th>Condition</th>
<th>NON-CARRIER</th>
<th>9p21 CARRIER</th>
<th>9p21 CARRIER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27% population</td>
<td><em>heterozygous 50% population</em></td>
<td><em>homozygous 23% population</em></td>
</tr>
<tr>
<td>Myocardial infarction/cardiovascular disease</td>
<td>no increased risk</td>
<td>up to 1.26X increased risk</td>
<td>up to 1.64X increased risk</td>
</tr>
<tr>
<td>Early myocardial infarction</td>
<td>no increased risk</td>
<td>up to 1.49X increased risk</td>
<td>up to 2.02X increased risk</td>
</tr>
<tr>
<td>Men &lt;50 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women &lt;60 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>no increased risk</td>
<td>up to 1.36X increased risk</td>
<td>up to 1.74X increased risk</td>
</tr>
</tbody>
</table>
Haptoglobin (Hp)

- Hp is an acute phase protein synthesized primarily by the liver in response to inflammatory cytokines.
- Hp binds oxygenated, free hemoglobin (Hb) with stabilization of the heme iron within Hb thus preventing oxidative tissue damage.
- The Hp-Hb complex is rapidly removed from the circulation via a monocyte-macrophage cell surface scavenger receptor.
- 3 Hp polymorphism in humans: 1-1, 1-2, and 2-2; antioxidant potency 1-1 > 1-2 > 2-2 supporting a link between Hp polymorphism and broad range of pathologic conditions.
- Diabetes individuals with Hp 2-2 have a greater risk of microvascular and macrovascular disease when compared to individuals with Hp 1-1 and Hp 1-2.
Structural Differences and Subunit Arrangements of Haptoglobin Phenotypes Influence Antioxidant Potency

Clin Chem 2008;54:697-704
Hemoglobin-Haptoglobin 2-2 Increases Oxidized LDL -> Atherosclerosis

HO-1 (heme oxygenase-1)

Biochemistry 2004;43:3899-3906
Vitamin E Supplementation Reduces CVD in Patients with Diabetes and Haptoglobin 2-2 Genotype

Women’s Health Study: Vitamin E ↑ CVD in Hp 1-1 and Hp 1-2 and ↓ CVD in Hp 2-2

<table>
<thead>
<tr>
<th></th>
<th>Hp 1-1</th>
<th>Hp 1-2</th>
<th>Hp 2-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (Vitamin E /Placebo)</td>
<td>112 (56/56)</td>
<td>332 (159/173)</td>
<td>277(146/131)</td>
</tr>
<tr>
<td>Absolute events n (% Total CVD)</td>
<td>12 (21.4%) vs. 10 (17.9%) Increase 19.6%</td>
<td>37 (23.3%) vs. 30 (17.3%) Increase 34.7%</td>
<td>31 (21.1% vs. 31 (23.7%) Decrease 10.5%</td>
</tr>
<tr>
<td>Adjusted total CVD HR [95% CI]</td>
<td>1.192 [0.456-3.117]</td>
<td>1.254 [0.766-2.055]</td>
<td>0.857 [0.511-1.435]</td>
</tr>
</tbody>
</table>

May help explain why some studies have shown a benefit with vitamin E and others have not (or harmful)

Atherosclerosis 2010;211:25–27
Pre-Haptoglobin 1 and 2 and Zonulin

Hp 1-1 No copies of Zonulin gene
Hp 1-2 One copy of Zonulin gene
Hp 2-2 Two copies of Zonulin gene
Zonulin: A Physiological Modulator of Tight Junctions (TJ), as Prehaptoglobin-2

- discovery of Vibrio cholerae zonula occludens toxin (Zot), a toxin that increases TJ permeability, led to the identification of its eukaryotic counterpart, zonulin
- several cancers and diseases of the neurologic system are associated with zonulin
- zonulin (pre-HP2) is a multifunctional protein
  --- intact single-chain precursor regulates intestinal permeability by transactivation epidermal growth factor receptor (EGFR)
  --- cleaved 2-chain form acts as a hemoglobin scavenger
- zonulin increases intestinal permeability and is overexpressed in autoimmune disorders in which TJ dysfunction is central, including type 1 diabetes and celiac disease
Key Components of the Intestinal Barrier

Tight Junction = Zonulin!!

Diseases Associated with Zonulin

Major diseases associated to Zonulin (Pre-HP2)

AUTOIMMUNE DISEASES
- Ankylosing spondylitis.
- Celiac disease
- Inflammatory bowel disease (Crohn’s disease)
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Type 1 diabetes

CANCERS
- Brain cancers (gliomas)
- Breast cancer
- Lung adenocarcinoma
- Ovarian cancer
- Pancreatic cancer

DISEASES OF THE NERVOUS SYSTEM
- Chronic inflammatory demyelinating polyneuropathy (CIDP)
- Multiple sclerosis (Autoimmune disease?)
- Schizophrenia (Autoimmune disease?)

Major diseases associated to Chromosome 16

AUTOIMMUNE DISEASES
- Adult polycystic kidney disease
- Inflammatory bowel diseases (NOD2 locus)
- Systemic lupus erythematosus
- Type 1 diabetes
- Rheumatoid arthritis

CANCERS
- Acute nonlymphocytic leukemia
- Breast cancer
- Fanconi’s anemia
- Lymphoma, diffuse large B-cell
- Myeloid leukemia, acute
- Prostate cancers

DISEASES OF THE NERVOUS SYSTEM
- Batten’s disease (juvenile onset neurodegenerative disorder)
- Lou Gehrig’s disease
- Leukodystrophy
- Multiple sclerosis
- Autism
Factor V Leiden

- Single point mutation in the F5 gene, G1691A arginine -> glutamine; named after the city Leiden (Netherlands), where it was 1st identified in 1994
- Most common inherited coagulation disorder in U.S. 5% Caucasians; 2% Hispanics; 1.2% African Americans; <0.5% Asian Americans
- Heterozygous = 3-8 fold increase risk of thrombosis
- Homozygous = 30-140 increase risk of thrombosis
- Overproduction of thrombin = excess fibrin formation -> excess clotting -> ↑DVT and PE
- Contraceptives and HRT should not be prescribed

Excess Venous Thromboembolism Events (VTE) Attributable to Oral Contraceptives and Hormone Replacement Therapy in Women with Factor V Leiden
Additional Genetic Testing for Certain Individuals

- LPA-aspirin genotype (rs3798220) and LPA-Intron 25 (rs10455872) - If Lp(a) is increased > 30 mg/dL
- MyPerioID and MyPerioPATH - periodontal disease
- ABO blood group - O lowest CVD risk
- Prothrombin Mutation - coagulation disorder
- CYP2C19*2*3 - Plavix poor metabolizer
- CYP2CA9*17* - Plavix rapid metabolizer
- MTHFR677 and MTHFR1298 - folate metabolism
- 4q25-AF Risk genotype - atrial fibrillation
- SLCO1B1 genotype - statin myopathy
Apo(a) is a large glycoprotein comprised of loop-like repeating units called kringles (tri-looped structure with 3 intramolecular disulfide bonds – resembling a Danish pretzel).

Apo(a) is linked by a disulfide bond to an LDL particle, producing a composite particle known as Lp(a).

The LPA gene encodes for the apo(a), a component of Lp(a).

Carriers of the LPA variant alleles have higher Lp(a) levels and a higher risk of atherosclerosis.

Lp(a) is potentially pro-thrombotic since it interferes with the action of plasminogen.
LPA Genotyping

- Two single nucleotide polymorphisms rs3798220 (LPA-aspirin genotype) and rs10455872 (LPA-Intron 25 genotype) associated with increased risk of CVD

- Lp(a) levels increased based on variant alleles:
  - No variant: 17 mg/dL
  - 1 variant: 60 mg/dL
  - 2 variants: >100 mg/dL

Women’s Health Study: LPA Variant and Aspirin Treatment—Carriers Treated with Aspirin Ameliorated ↑ Risk

*NNT to prevent 1 major CVD event was 37 for carriers and 625 for noncarriers; ~15 times more bleeds for each CVD event prevented in noncarriers than in carriers

Circ Cardiovasc Genet 2011;4:565-573
MyPerioID and Risk of Periodontal Inflammation

Interleukin 6 Genotypes
G/G (high risk)
G/C (moderate risk)
C/C (low risk)

Periodontal Inflammation Risk
LOW

Results:
MyPerioID Genotype C/C

Interpretation:
This individual's interleukin 6 genotype (IL6) is C/C. The MyPerioID result indicates your patient does not have an increased risk for periodontal inflammation due to the genetic variation examined in this test.

Significance: This individual's interleukin 6 (IL6) genotype is C/C and therefore is not at increased risk for periodontal disease by overproduction of interleukin-6 (IL-6) cytokine in the presence of bacteria. This individual's result does not rule out all risk for periodontal disease.

Risk: Studies demonstrate that carriers of the C allele are significantly less affected by periodontal disease when compared with carriers of the G allele. However, some patients may be MyPerioID low risk and have periodontal disease due to other risk factors.

Consider: IL-6 is a potent stimulator of osteoclast differentiation and bone resorption, is an inhibitor of bone formation, and overproduction has been implicated in systemic diseases such as juvenile chronic arthritis, rheumatoid arthritis, osteoporosis, Paget's disease and Sjogren's syndrome. The MyPerioID test assesses one of several risk factors that should be included in an overall evaluation of periodontal disease. Specific bacteria are associated with the initiation of the periodontal disease. Additional risk factors including other genetic markers, smoking, diabetes, and oral hygiene have an amplifying effect on disease progression and duration. The incidence of IL6 genotypes is reported to vary by ethnicity. Additional testing, such as MyPerioPath, may be considered if not already performed.
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Result</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porphyromonas gingivalis</em></td>
<td>Low</td>
<td>Very strong association with PD: Transmissible, tissue invasive, and pathogenic at relatively low bacterial counts. Associated with aggressive forms of disease.</td>
</tr>
<tr>
<td><em>Tannerella forsythia</em></td>
<td>Low</td>
<td>Very strong association with PD: common pathogen associated with refractory periodontitis. Strongly related to increasing pocket depths.</td>
</tr>
<tr>
<td><em>Prevotella intermedia</em></td>
<td>Low</td>
<td>Strong association with PD: virulent properties similar to Pg, often seen in refractory disease.</td>
</tr>
<tr>
<td><em>Capnocytophaga species</em> (gingivalis, ochracea, sputigena)</td>
<td>Low</td>
<td>Some association with PD: Frequently found in gingivitis. Often found in association with other periodontal pathogens. May increase temporarily following active therapy.</td>
</tr>
</tbody>
</table>
Inflammatory Biomarkers and Atherosclerosis

- F₂-Isoprostanes (F₂-IsopS)
- Oxidized LDL
- High sensitivity C-reactive protein (hs-CRP)
- Urine albumin creatinine ratio (UACR) + hyperfiltration!!
- Myeloperoxidase (MPO)
- Lipoprotein-associated phospholipase A2 (Lp-PLA₂) - Mass and Activity
- Homocysteine
Inflammatory Biomarkers and Atherosclerosis
F₂-Isoprostanes are an Index of Oxidant Stress in Humans

- Oxidative reactions play a significant role in many biological conditions including chronic diseases such as atherosclerosis
- F₂-isoprostanes are formed *in vivo* from the reaction of oxygen free radicals with arachidonic acid and have been found in oxidized LDL particles and atherosclerotic plaques
- Risk factors for atherosclerosis like obesity, smoking and diabetes are associated with increased levels of F₂-isoprostanes in humans
Increase in $F_2$-Isoprostanes Correlates with Body Mass Index

![Graph showing the correlation between BMI and $F_2$-Isoprostanes](image)

- P-value: $p<0.001$
F₂-Isoprostane Formation is Markedly Increased in Cigarette Smokers and Abstinence Decreases Levels

Relationship Between OXIDIZED LDL Cholesterol and Coronary Artery Disease

Percentage of Coronary Artery Disease in Patients

Oxidized LDL Cholesterol (U/l)

- 13% for 13 - 47 U/l
- 35% for 48 - 58 U/l
- 51% for 59 - 68 U/l
- 68% for 69 - 80 U/l
- 93% for 81 - 152 U/l

www.laddmcnamara.net
Mendelian Randomization and CVD-

hs-CRP NOT a Player

Eur Heart J 2014;35:1917-1924
Metabolic Syndrome and hs-CRP: More Components Higher hs-CRP Level

Circulation 2003;107:391-397
eGFR (<60 >105) and UACR > 5 Associated ↑ CV Mortality, ↑ CHD, Stroke, and Heart Failure

Hyperfiltration

HR: 1.0

eGFR
95 mL/min/1.73 m²

UACR

HR: 1.0

5 mg/g creatinine

Lancet Diabetes Endocrinol 2015;3:514-525
Hyperfiltration-Early Sign of Hypertension and Diabetes
Increased All-Cause Morality when the Albumin Creatinine Ratio is > 5
Linear Relationship: CV Mortality and Heart Failure with Urine Albumin Creatinine Ratio
Changes in Albuminuria Predict Major Clinical Outcomes in Diabetes

Diabetes Care 2017 https://doi.org/10.2337/dc17-1467
Empagliflozin Reverses the Initial Decline in eGFR

## Definitions of “Albuminuria”

<table>
<thead>
<tr>
<th>Urine albumin/creatinine ratio ‘UACR’ (mg/g creatinine)</th>
<th>Normo-albuminuria</th>
<th>Micro-albuminuria</th>
<th>Macro-albuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>30 - 300</td>
<td>&gt; 300</td>
<td></td>
</tr>
</tbody>
</table>

Diabetes Care 2004;27:S79-S83
Urine Albumin Creatinine Ratio (UACR)* and CVD Risk

Risk when UACR > 7.5 in women and > 4.0 men

<table>
<thead>
<tr>
<th>End Point</th>
<th>Hazard ratio</th>
<th>“p”</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV event</td>
<td>2.92</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*simple, inexpensive, independent predictor of CVD

Circulation 2005;112:969-975
Urine Albumin/Creatinine Ratio (UACR)

< 7.5 Women
< 4.0 Men
Estimated GFR (eGFR)

Normal: 95 (ML/MIN1.73M2)
Abnormal: <60/105
Myeloperoxidase (MPO)

MPO is elevated in 2 of every 50 patients
Myeloperoxidase (MPO)

- Member of the heme peroxidase family; stored in granules in leukocytes; secreted during leukocyte activation; important in innate infectious disease host defense
- Interacts with hydrogen peroxide and chloride to generate a powerful oxidant hypochlorous acid (HOCL) = BLEACH!!
- HOCL selectively targets nitrogen species to generate nitrotyrosine; HOCL also interacts with tyrosine residues to generate chlortyrosine
- ApoA-I, the primary protein that makes up ~75% of HDL particles, is oxidized by nitrotyrosine and chlortyrosine at Trp72, and such oxidation impairs the cardioprotective functions of HDL

Nat Med 2014;18:193-203
Model of Bidirectional Conversion of HDL from Anti-inflammatory (a) to Proinflammatory (b)
Lipoprotein-Associated Phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) Binds to ApoB on Oxidized LDL Promoting an Inflammatory Response
Darapladib: a Selective Lp-PLA$_2$ Inhibitor Failed to Meet Primary Endpoints in Two Key Clinical Trails

- Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy Trial (STABILITY)$^1$
- The Stabilization Of pLaques usIng Darapladib (SOLID)-TIMI 52 Trial$^2$*

*The baseline total cholesterol/HDL ratio was 3.4; LDL-C was 75, and Lp-PLA$_2$ was 172.5 (normal <200) so no surprise the studies did not meet their primary endpoints.

### Homocysteine and CVD NHANES III Population

<table>
<thead>
<tr>
<th>Homocysteine Level, μmol/l</th>
<th>All CVD or Hard CHD Events, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>All CVD events*</td>
<td></td>
</tr>
<tr>
<td>As a continuous variable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>As a categorical variable</td>
<td></td>
</tr>
<tr>
<td>I (n = 4,430)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>II (n = 1,804)</td>
<td>10-14.9</td>
</tr>
<tr>
<td>III (n = 563)</td>
<td>≥15</td>
</tr>
<tr>
<td>Hard CHD events*</td>
<td></td>
</tr>
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<td>As a continuous variable</td>
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</table>
Pro-BNP Derived Peptides: NT-proBNP and BNP

\[ t_{1/2} = 60 - 120 \text{ minutes} \quad t_{1/2} = 18 \text{ minutes} \]
NT-proBNP Strong Predictor of Heart Failure in patients with Type 2 Diabetes

![Graph showing hazard ratio with increasing fifths of NT-proBNP (pg/mL)]

Fifths of NT-proBNP (pg/mL):
- 1: 2.5–24
- 2: 25–59
- 3: 60–116
- 4: 117–255
- 5: 256.0–35,000

Hazard Ratio (95% CI):
- P for trend < 0.001

Diabetes Care 2017;40:1203–1209 The Advance Trial
Valsartan + sacubitril, a neprilysin inhibitor (Entresto)
Neprilysin Improves Glycemic Control by Multiple Mechanisms

Sacubitril/valsartan

- Neprilysin inhibition
  - ↑Natriuretic peptides
    - ↑Insulin sensitivity
    - ↑Lipid mobilisation
    - ↑Insulin metabolism
    - ↑Adiponectin release
    - ↓Blood glucose levels
    - ↑Postprandial lipid oxidation
    - ↑Muscular oxidative capacity
  - ↑Bradykinin
    - ↑Insulin sensitivity
    - attenuated lipolysis
  - ↑GLP-1
  - ↑Skeletal muscle cGMP
    - Facilitated lipolysis vasodilation
- ↓DPP-4 activity
  - ↑β-cell function

AT₁-receptor blockade

- ↑Insulin sensitivity

Lancet Diabetes Endocrinol 2017;5:314-315
Empagliflozin ↓ Cytoplasmic Na\(^+\), Diastolic and Systolic Ca\(^{++}\) and ↑ Mitochondrial Ca\(^{++}\)

Net impact: improved cardiac mitochondrial function
Happy Heart

NT-proBNP <125 pg/mL
Canakinumab (Interleukin-1β antibody) Significantly Lower Rate of Recurrent Cardiovascular Events vs. Placebo

NEJM 2017 DOI: 10.1056/NEJMoa1707914 NO significant difference all-cause mortality
Obstructive Sleep Apnea: Intermittent Hypoxia and Sleep Fragmentation Promotes Insulin resistance

Physiol Rev 2010;90:47-112
Obstructive Sleep Apnea-KISS
(Keep it Simple Stupid)

- Does the bed partner complain of snoring or any odd breathing patterns during the night
- Any complaints of being tired, sleepy or fatigued during the day
- Any comorbid disorders that are associated with sleep apnea
- Obesity

Yes to 2 or more warrants a sleep study to r/o OSA
"I'm going to hibernate in another cave. You have sleep apnea and your snoring kept me up all of last winter."
Telomers and Micronutrients

- **Calcium**
  - Required cofactor to prevent DNA replication errors.

- **Manganese**
  - Required cofactor in Mn superoxide dismutase, a deficiency in which decreases telomerase activity.

- **Vitamin D**
  - Positively associated with telomere length due to its anti-inflammatory role.

- **Vitamin E**
  - Enhances DNA repair as well as removal of damaged DNA; shown in vitro to restore telomere length on human cells.

- **Folate**
  - Influences telomere length via DNA methylation.

- **B3**
  - Extends lifespan of human cells in vitro; slows telomere attrition rate by reducing reactive oxygen species in mitochondria.

- **B2, B6 and B12**
  - Crucial for proper DNA methylation.

- **Cysteine**
  - Stem cell treatment with N-acetyl cysteine corrects DNA damage in telomeres.

- **Zinc**
  - Important cofactor for DNA repair enzymes; key role in regulating inflammation.

- **Copper**
  - Key cofactor in the potent antioxidant superoxide dismutase that is known to protect telomeres.

- **Magnesium**
  - Induced deficiency shortened telomeres in rat livers; regulates chromosome separation in cell replication.

- **Vitamin C**
  - Protects DNA from oxidation. In vitro studies show it slows down age-related telomere shortening in human skin cells.

- **Glutathione**
  - Interference of glutathione dependent antioxidant defenses accelerates telomere erosion.

- **Selenium**
  - In vitro supplementation extended telomere length in liver cells; selenoproteins protect DNA.
Telomere Length Dr. Lardinois!

Telomere Test Results

Patient Telomere Score: 6.02
Percentile relative to patient age and population: 32%
## Cardiovascular Outcome Trials

<table>
<thead>
<tr>
<th>Drug</th>
<th>MACE</th>
<th>CV Death</th>
<th>Non-Fatal MI</th>
<th>Non-Fatal Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empagliflozin SGLT2i</td>
<td>√√</td>
<td>√√</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Canagliflozin SGLT2i</td>
<td>√√</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Liraglutide GLP-1A</td>
<td>√√</td>
<td>√√</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Semaglutide GLP-1A</td>
<td>√√</td>
<td>↔</td>
<td>↔</td>
<td>√√</td>
</tr>
<tr>
<td>Exenatide GLP-1A</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Lixisenatide GLP-1A</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>ITCA 650 GLP-1A</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Saxagliptin DPP-IVi</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Sitagliptin DPP-IVi</td>
<td>↔</td>
<td>↔</td>
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<tr>
<td>Alogliptin DPP-IVi</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Acarbose α-glucosidasei</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
</tbody>
</table>

√√ = superiority  ↔ = non-inferiority
Paradigm Shift: New Algorithm to Treat Type 2 Diabetes

**eGFR >30

***eGFR >45

J Clin Lipidol 2017;11:1126–1133
Cardiovascular Disease, Diabetes, Atherosclerosis-Closing Remarks

- Atherosclerosis continues to be the number one cause of death in the USA.
- Current standard of care using risk factor identification and modification fails to identify many patients that are at risk of developing heart disease and stroke.
- Non-invasive testing, genetics, and inflammatory markers enhance the ability to identify disease (atherosclerosis) earlier.
- When disease is found, the ‘root’ causes must be identified and treated.
- A paradigm shift focusing on disease (atherosclerosis) is mandated to reduce the high rate of recurrence.
Non-Invasive, Advanced Lipid and Genetic Testing for CVD

- CardioRisk [http://www.cardiorisk.us](http://www.cardiorisk.us)
- ClevelandHeartLab [http://www.clevelandheartlab.com/](http://www.clevelandheartlab.com/)
- LabCorp [https://www.labcorp.com/](https://www.labcorp.com/)
- SpectraCell Laboratories [https://www.spectracell.com/](https://www.spectracell.com/)
- TrueHealthDiagnostic [https://my.truehealthdiag.com/](https://my.truehealthdiag.com/)

Dr. Lardinois declares NO financial interests in any of these companies.
OUR HEARTS AND PRAYERS GO OUT TO VEGAS
Thank you for your attention!

Happy to Answer Any QUESTIONS?